

## Supporting Information

Directed Evolution and Biophysical Characterization of  
a Full-length, Soluble, Human Caveolin-1 Variant\*

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\*Running title: *Evolution and characterization of a membrane protein*

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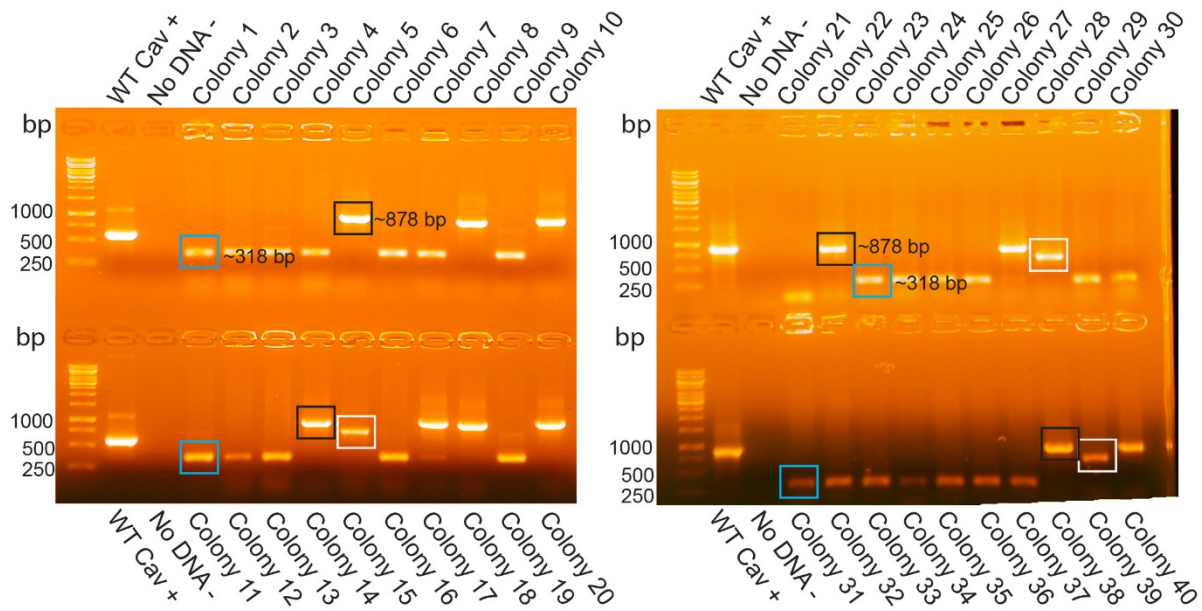


Fig S1. Representative colony PCR of round 4 selectants after sub-cloning into the pET28c vector. The resultant DNA was transformed into *E. coli* BL21 (DE3) cells. The presence of cav DNA was examined by colony PCR and imaged on a 1.0% agarose gel. Three outcomes were observed. The colonies contained DNA encoding (1) self-ligated pET28c vector, (2) a truncated, or (3) a full-length cav variant. These three scenarios are represented by the blue, white, or black boxes respectively. Each gel included positive and negative PCR controls of WT cav DNA and no DNA respectively. Approximately 160 total colonies were examined (data not shown for all).

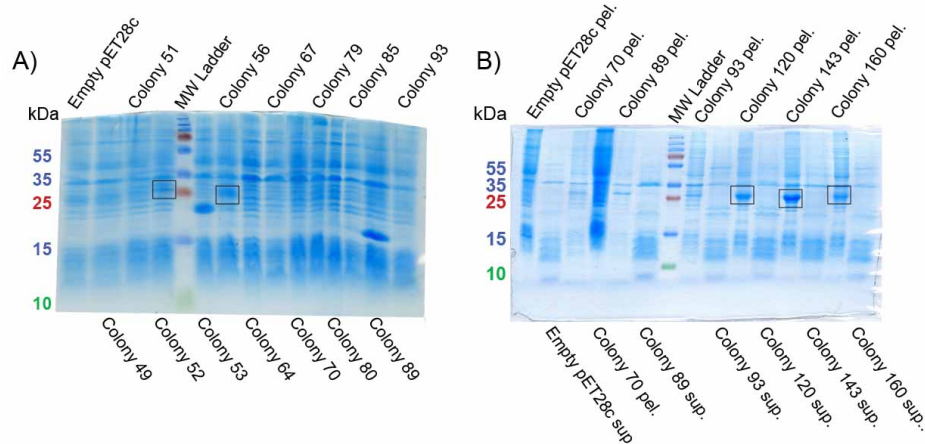


Fig. S2. Screening for protein overexpression of round 4 variants. Colonies with full-length cav DNA (from Fig S1) were screened for protein overexpression as analyzed by SDS-PAGE (15%). A) Small, 1 mL cultures were grown and induced for protein expression. B) Larger, 100 mL cultures were grown for any colony with potential protein overexpression in the small cultures. After overexpression, larger cultures were lysed by sonication. Their insoluble pellet (pel.) and soluble supernatant (sup.) fractions were analyzed separately. The expected size of full-length caveolin is ~ 25 kDa (black boxes). The highest expressing variant (panel B, colony 143) was later termed cav<sub>sol</sub> and was used for further experiments. In total, ~50 colonies were overexpressed as small cultures and ~20 were overexpressed as larger cultures (data not shown for all).

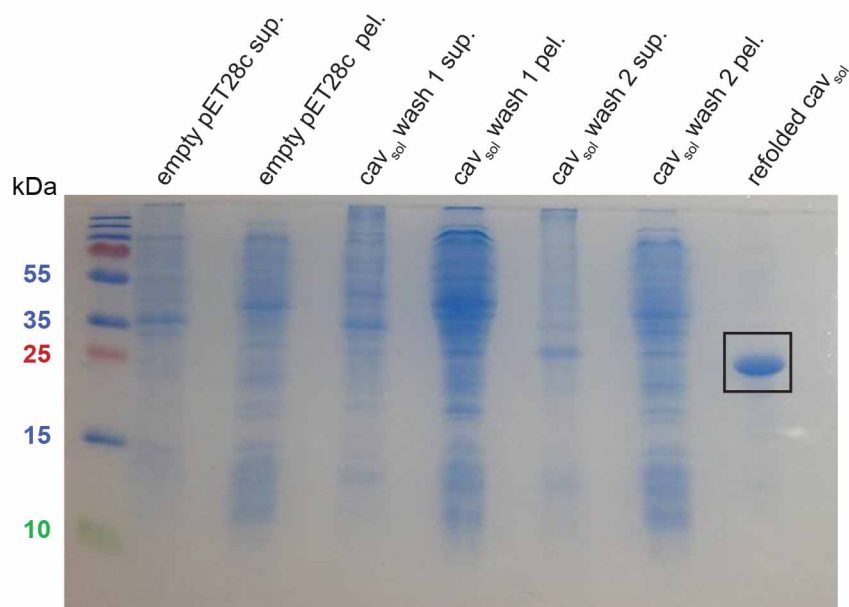


Fig. S3. Protein overexpression and purification of  $cav_{sol}$ .  $Cav_{sol}$  was overexpressed as described in the materials and methods. After purification using  $Ni^{2+}$ -charged IMAC resin and FPLC-assisted size exclusion chromatography,  $cav_{sol}$  was refolded and analyzed for purity (black box). The homogeneity for this preparation of  $cav_{sol}$  is estimated to be 95% by ImageJ software<sup>1</sup>.

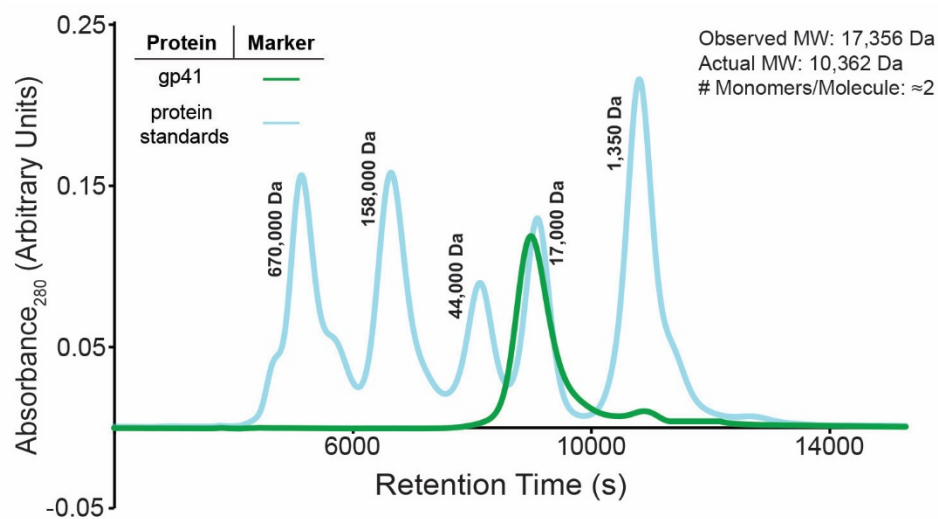


Fig S4. Size exclusion chromatography of gp41. HIV gp41 was analyzed by size exclusion chromatography (SEC) to increase the homogeneity of the protein for subsequent use and also to determine the oligomeric state of the protein. The SEC spectrum of gp41 (green) shows the protein largely forms dimers. Estimating the molecular weight of the gp41 using molecular weight standards confirms this oligomeric state (blue).

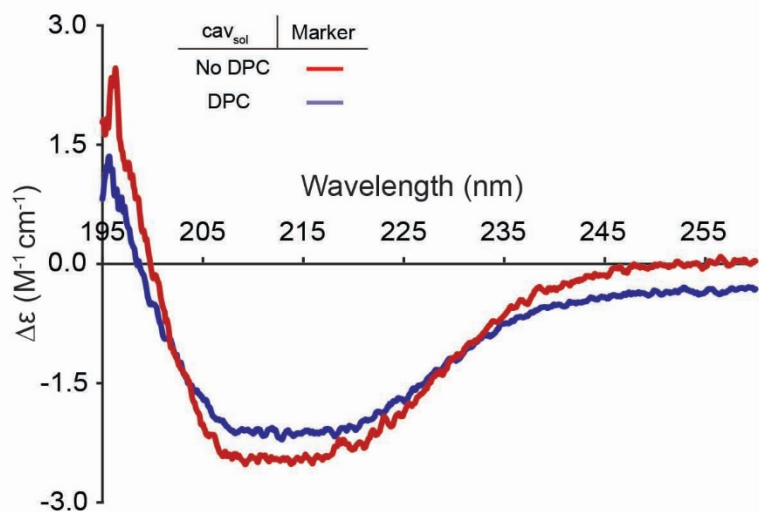


Fig S5. CD analysis of  $cav_{sol}$  in the presence and absence of dodecylphosphatidylcholine micelles (DPC). The CD spectrum of  $cav_{sol}$  was obtained first without the presence of DPC (red) and then after the addition of 50 mM DPC (blue). The DPC-doped sample was allowed to incubate for 1 h before measuring the CD spectrum.

Table S1. Differing cav oligomerizatic states in literature.

Number of Monomers / Complex	Isolated From	Method of Determination
9,18,27 <sup>2</sup>	Canine Brain	SDS-PAGE
144 ± 39 <sup>3</sup>	MEF cells	Fluorescent Labeling/Imaging
9,14-16 <sup>4</sup>	MDCK cells	Gradient Centrifugation, SDS-PAGE
≈88-133 <sup>5</sup>	MDCK cells	Gradient Centrifugation
≈7-14, 160 <sup>6</sup>	CV1 cells	Gradient Centrifugation
7 <sup>7</sup>	Overexpressed in <i>E. coli</i> (truncated)	Gel Filtration Chromatography / Electron Microscopy

#### Supplemental Information References

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